Patent Application
Attorney's Docket No. 010055-134

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	
Simon C. BURTON et al.	Group Art Unit: 1651
Application No.: 08/468,610	Examiner: Jon P. Weber, Ph.D.
Filed: June 6, 1995	
For: CHROMATOGRAPHIC RESINS AND METHODS FOR USING SAME	

DECLARATION UNDER 37 C.F.R. § 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Simon C. BURTON, do hereby declare:

1. THAT, I have received a Bachelor of Veterinary Science from Massey University in 1987, a Postgraduate Diploma of Science in Biochemistry from Massey University in 1991 and a Ph.D. in Biochemistry from Massey University in 1996.

2. THAT, I am an employee of Massey University (hereinafter "Massey"), where I have worked since 1988. I am biochemist by training and am currently a Research Officer in the Institute of Fundamental Sciences at Massey. My current research focuses on enzyme purification and immobilization, matrix development, analysis and screening. From 1988 to 1997, I held the position of Research Technician, Junior Research Office, and Research Officer in the Department of Chemistry/Biochemistry at Massey. During this time, I performed research in the chromatographic purification of proteins, especially industrial enzymes and immunoglobulins, and matrix modification chemistry. This research had special emphasis on inexpensive, industrial protein recovery and one step

purification methods. More specifically, I have carried out and directed experimental work involving the separation and purification of proteins using ion exchange chromatography and other forms of chromatography, which requires a working knowledge of how to prepare, adsorb and elute proteins from ion exchange columns.

3. THAT, a copy of my Curriculum Vitae is attached hereto as Appendix A.

4. THAT, I am one of the joint inventors of the subject matter disclosed and claimed in the above-referenced application, and I have reviewed and am familiar with the contents of U.S. Patent Application Serial No. 08/468,610 (hereinafter "'610 patent application") including the currently pending claims.

5. THAT, the invention in the '610 patent application relates to complexes of chromatographic resins with proteins and peptides. In particular, the chromatographic resins are useful for the binding of a target protein or peptide from an aqueous medium that has either a high or low ionic strength. Central to the claimed invention is the use of an electrostatically uncharged resin at the pH where the target protein or peptide is bound to the resin which has a pH in the range of from 5 to 9 when the aqueous medium has either a high or low ionic strength. In addition, the resin is selected such that it contains an electrostatic charge at the pH where the protein or peptide is desorbed from the resin, wherein the desorption occurs by a change in the pH from the binding pH.

6. THAT, I have reviewed and am familiar with the Office Action dated September 28, 2001. I have also reviewed and am familiar with the Examiner's rejection of the claims alleging that such claims are purportedly anticipated by Boardman et al., *Nature*, 171:208-210 (1953) (hereinafter "Boardman").

7. THAT, I have reviewed and am familiar with the Examiner's rejection of the claims alleging that such claims are purportedly obvious over Boardman, Sasaki et al., *J.*

Biochem., 86:1537-1548 (1979) ("Sasaki 1979") and Sasaki et al., *J. Biochem.*, 91:1551-1561 (1982) ("Sasaki 1982") in view of Kunin, Ion Exchange Resins, 34-39 (John Wiley & Sons, Inc., Interscience 1958) ("Kunin"), Topp et al., *J. Chem. Soc.*, Pt. 2:3299-3303 (1949) ("Topp"), Kitchener, Ion Exchangers In Organic and Biochemistry, 63-64 (Calmon and Kressman eds., Interscience Publishers, Inc. 1957) ("Kitchener") and Guthrie, Ion Exchangers In Organic and Biochemistry, 558-559 (Calmon and Kressman eds., Interscience Publishers, Inc. 1957) ("Guthrie").

8. THAT, I have reviewed and am familiar with the Reply of January 28, 2002, filed in response to this Office Action which includes the Declaration under 37 C.F.R. § 1.132 by Nathaniel T. Becker, one of the joint inventors of the subject matter disclosed and claimed in the present application (hereinafter the "Becker Declaration"). The Becker Declaration was filed as an attachment to the Reply of January 28, 2002.

9. THAT, I have reviewed and am familiar with the product literature regarding the synthetic cation exchange resin, Amberlite IRC-50, provided by the manufacturer, Rohm and Haas Co. (2000) (hereinafter "Rohm and Haas"). A copy of Rohm and Haas was attached to the Becker Declaration that was filed with the Reply of January 28, 2002.

10. THAT, subsequent to the Becker Declaration, I generated experimental titration data for the carboxylate Amberlite IRC-50 resin and the carboxylate Amberlite CG50 resin. The Amberlite CG50 resin is essentially equivalent to the Amberlite IRC-50 except that the bead size for the IRC-50 resin is several times greater. I have compared these experimental titration curves with theoretical titration curves calculated using the simple Henderson-Hasselbalch equation (pH=pKa - log(acid/base)). A copy of this data is attached hereto as Appendix B.

11. THAT, subsequent to the Becker Declaration, I compared the IRC150 titration curve as disclosed in the literature with theoretical titration curves calculated using the simple Henderson-Hasselbalch equation (pH=pKa - log(acid/base)). A copy of this data is attached hereto as Appendix C.

12. THAT, I have reviewed and am familiar with the Boardman, Sasaki 1979, Sasaki 1982, Kunin, Topp, Kitchener and Guthrie references cited in the Office Action of September 28, 2001. In relation to Boardman, the September 28, 2001, Office Action at page 2 contains the following statement:

> At a low pH the cation exchange media is uncharged and binds the proteins. As the pH is raised, the protein is eluted. Figure 1(a) illustrates the technique with cytochrome C on Amberlite IRC-50 [a cross-linked poly(methacrylic acid) with a capacity of 10 Meq/g]. At a pH value of 5, cytochrome C is tightly bound to the media whose carboxylic groups are said to be wholly uncharged.

13. THAT, in my opinion the Examiner's characterization of the carboxylate Amberlite IRC-50 resin is inaccurate. The Examiner appears to rely heavily on the pKa values of the carboxylate ion exchangers. In particular, the Examiner appears to quote pKas in water. However, the carboxylate titration data which I generated, as well as the titration curves shown in the cited art, clearly indicate that the pKa is irrelevant except for theoretical calculations of the percentage of protonated carboxyl groups using the Henderson-Hasselbalch equation. This equation states that the ratio of protonated to unprotonated carboxyl groups is 1 at pH equal to the pKa; 10 at pH 1 unit below the pKa; and 0.1 at pH 1 unit above the pKa. Thus, about 82% of the titration curve should lie between the values of 1 unit either side of the pKa. However, the titration curves in the above-cited references, in particular Kunin, Kitchener, Rohm and Haas, plus the carboxylate titration curve generated from my own data do not fit this. See, Appendix B, Sheet 2, chart; Appendix C, Chart 1. As can be seen, the titration range is broader, probably due to heterogeneity and neighboring group effects in a polyvalent species such as

an ion exchanger. Thus, I believe theoretical pKa data should be ignored in favor of experimental titration data.

14. THAT, in my opinion, the experimental titration curves make it evident that titration of protonated carboxylates starts before pH 5 even at very low ionic strength. The higher the salt concentration the earlier titration starts. Since the claims of the '937 patent application contain the limitations "uncharged" and "a high or low ionic strength." there is a requirement that the support be uncharged at high (or low) ionic strength. However, at high ionic strength, Boardman's data shows at least 20% carboxyl groups are unprotonated at pH 5. This is consistent with the experimental titration curve that I generated and with those equivalents found in the art references. See Appendix B. Thus, it appears that the percentage groups titrated at pH 5 is 20%. This clearly contradicts the Examiner's argument at page 2 of the Office Action wherein it is stated that "[a]t a pH value of 5, cytochrome C is tightly bound to the media whose carboxylic groups are said to be wholly uncharged." Therefore, it is my opinion that Boardman fails to demonstrate binding to an uncharged resin between pH 5 and 9, and Boardman fails to demonstrate binding at high or low ionic strength. Furthermore, the statement that "carboxylic groups are said to be wholly uncharged" appears to approach reality only in a distilled water titration. This is clearly not the case at high or low ionic strength as evident from the data in Boardman in 1M chloride; the data from the other cited references; the data from the manufacturers; and my own data. For instance, Kunin depicts titration from pH 3.0 in 1M NaCl. Kitchener likewise reports titration from pH 4.0 in 0.1 M NaCl. The cited Sasaki articles also indicate that a pH of 4.5 or less is required to completely protonate the carboxyl groups of the resins. The product literature as described in Rohm and Haas also reports titration between a pH of 2.5 to 4.0 In other words, it is clear that the carboxylate Amberlite IRC-50 becomes fully protonated (neutralized) only at a pH of between 2.5 and 4.0. This clearly contradicts the assertions made in the Office Action and it fails to meet the limitations of the claims.

15. THAT, in my opinion those skilled in the art would recognize that no basis is seen in the Office Action or in any of the references to conclude that a protein binds to an uncharged Amberlite IRC-50 carboxylate resin in the range of pH 5 to 9 at a high or low ionic strength.

16. I further declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 3/27/02 Signed: Simon C Burton

Simon C. Burton

APPENDIX A

CURRICULUM VITAE Simon Christopher Burton
c/o IFS, Massey University, Private Bag 11222
Palmerston North, New Zealand.
Work (+64) 63569099 (x3549)
S.C.Burton@massey.ac.nz

Academic Qualifications:

B.V.Sc. (veterinary science), Massey University, 1987.
Postgraduate Diploma of Science, Biochemistry, Massey University, 1991.
Ph.D. (Biochemistry), Massey University, 1996.

Employment History:

1988-1997 Research Technician, Junior Research Officer and Research Officer: Dept. of Chemistry/Biochemistry, Massey University. Responsible for research into chromatographic purification of proteins (especially industrial enzymes and immunoglobulins) and matrix modification chemistries. Special emphasis on inexpensive, industrial protein recovery and one step purification methods. Pilot scale studies (at Genencor International).

1998-2000 Postdoctoral Research Fellow, Centre for Bioprocess Engineering, Chemical Engineering, University of Birmingham. Carried out research into the purification of plasmid gene therapy vectors. In particular, development of novel matrices, designed for separation of nanoparticulate bioproducts and plasmid analysis methods for process development and monitoring.

2001-2002 Research officer, Institute of Fundamental Sciences, Massey University: Enzyme purification and immobilisation. Matrix development, analysis and screening.

Publications

[1] Haggarty, N.W., Burton, S.C., Hock, B.D. and Harding, D.R.K. (1990), "High Yield, Economic Recovery of Low Value Proteins" in Fermentation Technologies: Industrial Applications, Pak-Lam Yu (editor) 407-412.

[2] Burton, S.C., Haggarty N.W. and Harding, D.R.K. (1991) J. Chromatogr., 587, 271-275, "Efficient Substitution of Carbonyldiimidazole Activated Cellulose and Sepharose Matrices with Aminoacyl Spacer Arms".

[3] Haggarty, N. W., Campanella, M.E.P., Burton, S.C. and Harding, D.R.K., (1992), "Design of Bioaffinity Matrices for the Recovery of Valuable Enzymes from Various Biological Sources", in Proceedings of the Twenty-Seventh Meat Industry Research Conference, Hamilton, N.Z., 258-262.

[5] Burton, S.C., "Preparation of Chemically Modified Bead Cellulose Resins and their Application to Protein Purification", PhD Thesis, 1996, Massey University, Palmerston North, New Zealand.

[6] Burton, S.C., Haggarty N.W. and Harding, D.R.K. (1997) Biotechnol. Bioeng., 56, 45-55 "One step purification of chymosin by mixed mode chromatography".

[7] Burton, S.C. and Harding, D.R.K. (1997) J. Chromatogr., 775, 29-38, "Bifunctional etherification of a bead cellulose for ligand attachment with allyl bromide and allyl glycidyl ether".

[8] Burton, S.C. and Harding, D.R.K. (1997) J. Chromatogr., 775, 39-50, "High density ligand attachment to brominated allyl matrices and application to mixed mode chromatography of chymosin".

[9] Burton, S.C. and Harding, D.R.K. (1997) J. Chromatogr., 796, 273-282, "Preparation of chromatographic matrices by free radical addition ligand attachment to allyl groups".

[10] Burton, S.C. and Harding, D.R.K. (1998) J. Chromatogr. 814, 71-81, "Hydrophobic charge induction chromatography: salt independent protein adsorption and facile elution with aqueous buffers".

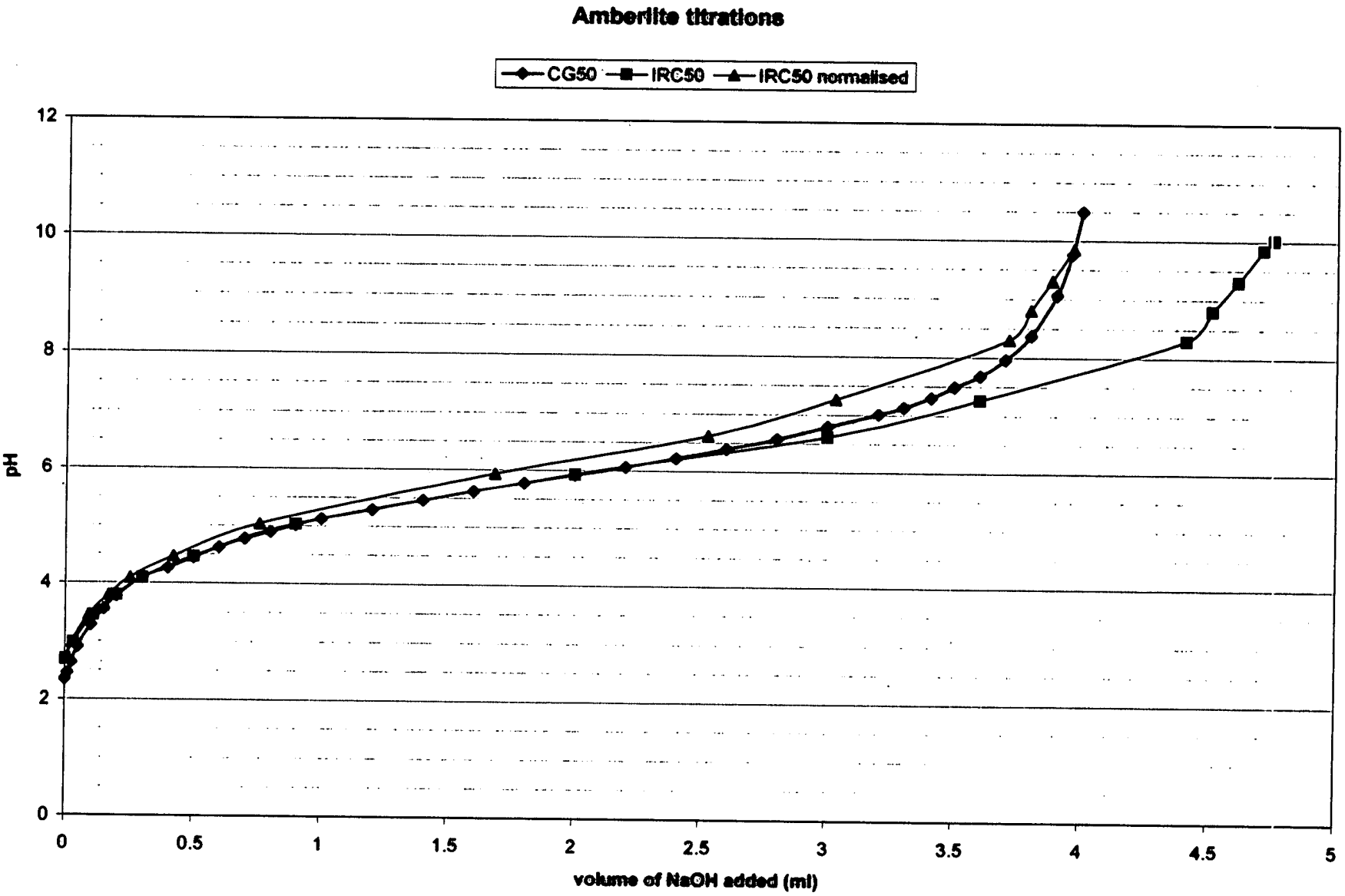
[11] Hamilton, G.E., Luechau, F., Burton, S.C. and Lyddiatt, A., J. Biotechnol., 79 (2000) 103-115, "Development of a mixed mode adsorption process for the direct product sequestration of an extracellular protease from microbial batch cultures".

[12] Zhang, Z., Burton, S., Williams, S., Thwaites, E. and Lyddiatt, A., Bioseparation 10 (1-3) (2001) 113-132, "Design and assembly of solid-phases for the effective recovery of nanoparticulate bioproducts in fluidised bed contactors".

[13] Burton, S.C. and Harding, D.R.K., J. Biochem. Biophys. Methods, 49 (1-3) (2001) 275-287, "Salt independent adsorption chromatography: new broad spectrum affinity methods for protein capture".

[14] Thwaites, E., Burton, S.C. and Lyddiatt, A., J. Chromatogr. A, 943(1) (2002) 77-90, "Impact of the physical and topographical characteristics of adsorbent solid phases upon the fluidised bed recovery of plasmid DNA from *E. coli* lysate".

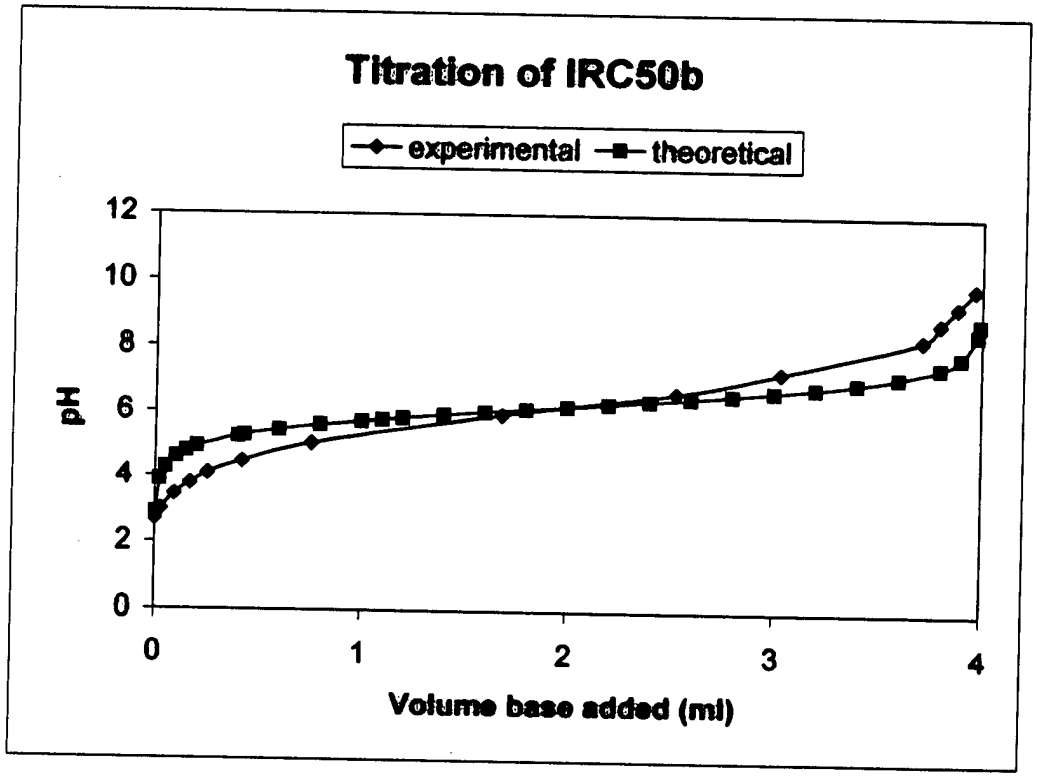
APPENDIX B


Amberlite titrations
CG50
IRC50
IRC50 normalised
12
10
8
6
4
2
0
pH
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
volume of NaOH added (ml)

Theoretical titration from H nd rson-Hasselbach

ba e	protonated	log(HA/A)	pH
0.002	3.998	3.300812794	2.90
0.02	3.98	2.298853076	3.90
0.048	3.952	1.915575699	4.28
0.1	3.9	1.591064607	4.61
0.148	3.852	1.415424563	4.78
0.2	3.8	1.278753601	4.92
0.4	3.6	0.954242509	5.25
0.43	3.57	0.919199761	5.28
0.6	3.4	0.753327667	5.45
0.8	3.2	0.602059991	5.60
1	3	0.477121255	5.72
1.1	2.9	0.421005313	5.78
1.2	2.8	0.367976785	5.83
1.4	2.6	0.268845312	5.93
1.6	2.4	0.176091259	6.02
1.8	2.2	0.087150176	6.11
2	2	0	6.20
2.2	1.8	-0.087150176	6.29
2.4	1.6	-0.176091259	6.38
2.6	1.4	-0.268845312	6.47
2.8	1.2	-0.367976785	6.57
3	1	-0.477121255	6.68
3.2	0.8	-0.602059991	6.80
3.4	0.6	-0.753327667	6.95
3.6	0.4	-0.954242509	7.15
3.8	0.2	-1.278753601	7.48
3.9	0.1	-1.591064607	7.79
3.98	0.02	-2.298853076	8.50
3.99	0.01	-2.600972896	8.80

A pKa of 6.2 has been estimated and used to generate the theoretical curve by Henderson Hasselbach calculation (data at left). Data (sheet3) for the experimental curve has been "normalised" from a total base addition of 4.75 to 4 ml



Experimental data for titration of 1g wet weight (0.392g dry) Amberlite CG50 in 5 ml of 1 M NaCl with 1M NaOH

base = volume of Convol NaOH added (ml)
Graphical depiction in Sheet1

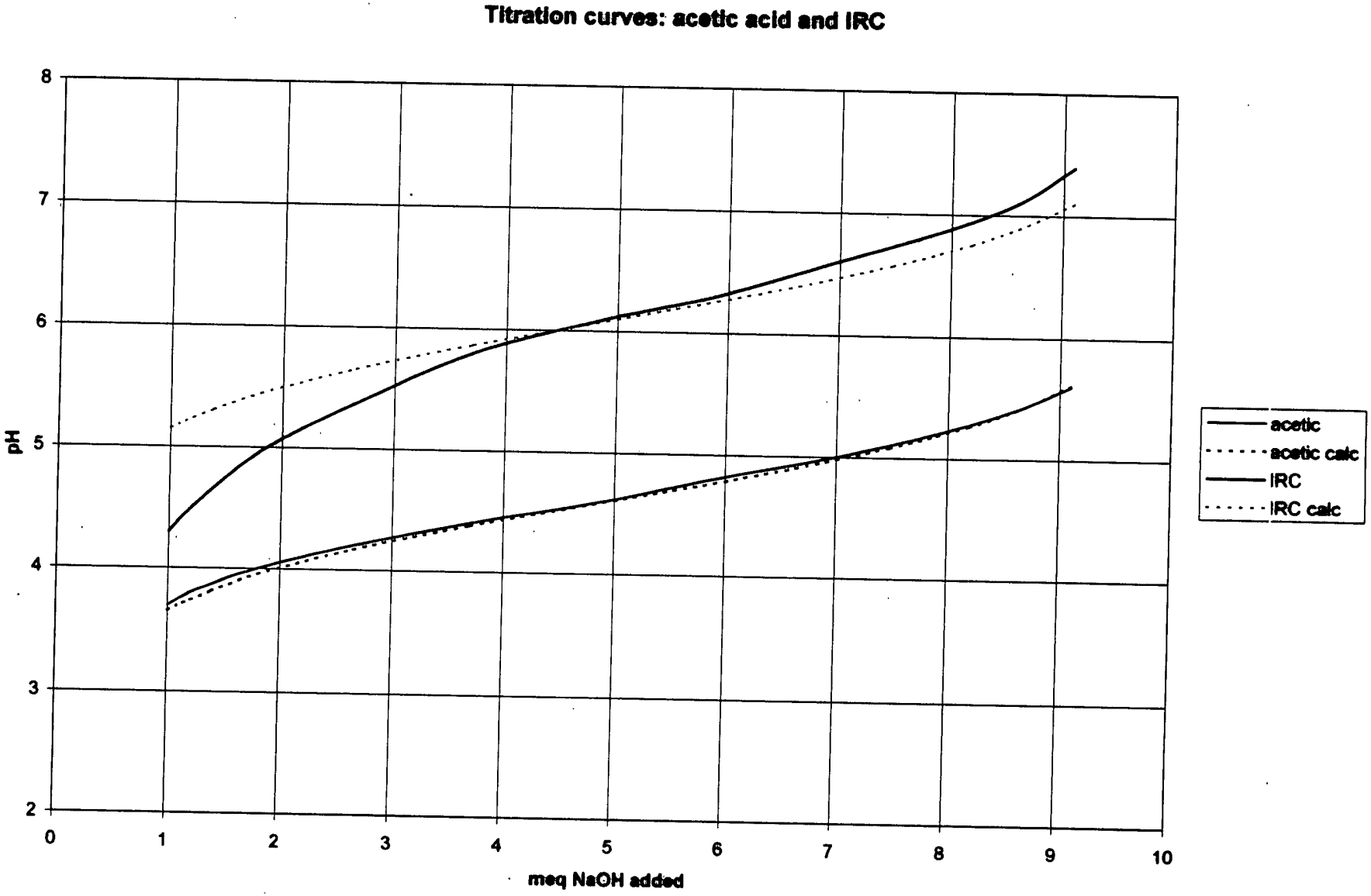
Column A base	Column B pH	Column C	Column D base	Column E pH	Column F
0	2.34		0	2.69	0
0	2.34		0.032	2.98	0.026947
0.01	2.45		0.109	3.45	0.091789
0.025	2.63		0.2	3.79	0.168421
0.05	2.9		0.3	4.09	0.252632
0.1	3.28		0.5	4.46	0.421053
0.15	3.55		0.9	5.03	0.757895
0.2	3.77		2	5.93	1.684211
0.3	4.09		3	6.61	2.526316
0.4	4.26		3.6	7.26	3.031579
0.5	4.44		4.41	8.3	3.713684
0.6	4.62		4.51	8.8	3.797895
0.7	4.77		4.61	9.3	3.882105
0.8	4.89		4.71	9.85	3.966316
0.9	5.01		4.75	10.02	4
1	5.12				
1.2	5.29				
1.4	5.46				
1.6	5.62				
1.8	5.77				
2	5.92				
2.2	6.06				
2.4	6.22				
2.6	6.39				
2.8	6.57				
3	6.79				
3.2	7.01				
3.3	7.13				
3.408	7.3				
3.5	7.49				
3.6	7.68				
3.7	7.96				
3.8	8.37				
3.9	9.06				
3.96	9.76				
4	10.49				

Column A is CG50 data
Column D is raw data for IRC50

Column F is IRC50 data normalise to a total base addition of 4 ml

APPENDIX C

Chart 1


Titration curves: acetic acid and IRC
pH
meq NaOH added
8
7
6
5
4
3
2
0
1
2
3
4
5
6
7
8
9
10
acetic
acetic calc
IRC
IRC calc

Column A NaOH	Column B Hac	Column C pH	Column D	Column E	Column F
1	10	3.7	3.66	4.3	5.15
1.2	10	3.8	3.74	4.5	5.23
1.8	10	4	3.95	4.96	5.44
2.65	10	4.2	4.17	5.37	5.66
3.7	10	4.4	4.38	5.8	5.87
4.9	10	4.6	4.59	6.1	6.08
5.9	10	4.8	4.77	6.3	6.26
7	10	5	4.98	6.6	6.47
7.9	10	5.2	5.19	6.85	6.68
8.6	10	5.4	5.40	7.1	6.89
9.1	10	5.6	5.61	7.4	7.10

equation used for columns D and F is: pH = pKa - log(HA/A-)

where pKa values used are 4.61 (acetic) and 6.1 (IRC)
HA = B#-A#
A- = A#

Acetic acid data is derived from buffer tables for 0.2M acetate buffer preparation,
Data for Biochemical Research, Dawson et al., Oxford Science Pub., 1986

Chart 1 shows good equivalence between pH from buffer tables and that calculated by the Henderson Hasselbach equation (acetic) but poor equivalence for IRC, which titrates over a broader pH range than predicted.